NTMC2T5: lipid transfer proteins at ER-chloroplast contact sites involved in plant stress.

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Chloroplasts are the site of fatty acid synthesis in plants; however, these fatty acids are assembled into glycerolipids at the ER. Later on, some of these ER-assembled glycerolipids will be transferred back to the chloroplasts to be further modified and to form part of the chloroplastic membranes. Previous reports have shown that under some abiotic stresses, these plastid membranes suffer a large lipid remodelling and new precursors massively need to be transported from the ER to the chloroplast or vice versa. It has been suggested that the newly synthetized ER lipids are delivered to chloroplast via a non-vesicular pathway, likely through lipid transport proteins (LTP). These LTP would be localized in membrane contact sites (MCS).

Some LTP at MCS contain particular domains, as the synaptotagmin-like mitochondrial lipid-binding (SMP) domain. We have studied the occurrence of SMP proteins in *A. thaliana* and *S. lycopersicum*. By using transient expression in *N. benthamiana* leaves and confocal microscopy, we have identified the NTMC2T5 family with two homologs in *A. thaliana* and only one in *S. lycopersicum.* They are anchored to the chloroplast outer membrane, and they interact *in trans* with the ER (ER-chloroplast MCS).

We have observed that clustering of chloroplasts around the nucleus occurred when we overexpressed these proteins and *Arabidopsis* double *knock-out* mutant for these proteins showed less chloroplasts attached to nuclei at control conditions. And, we have investigated the NTMC2T5 protein domains involved in this clustering.

Moreover, our analysis has demonstrated that *Arabidopsis* simple mutants show lower germination rates in media supplemented with NaCl and lower rates of expanded cotyledons in media supplemented with ABA. We have also performed biotinylation-based proximity labelling proteomics experiments in order to identify interactors of these proteins. Finally, we have performed lipidomic analysis to understand the role of these proteins.

Our results show the identification of a family of proteins localized at ER-chloroplast MCS, specifically interacting with the ER that compose the outer nuclear envelope. Our results suggest that these proteins might have a role in lipid transfer between these two organelles and that they might be involved in abiotic stress signalling through an ABA-dependent pathway.